VII.

CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF THE MUSEUM OF HARVARD UNIVERSITY.*

V. — ON THE ANATOMY AND DEVELOPMENT OF AGARUM TURNERI, Post. & Rupr.

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The family of Laminariaceæ includes several genera of large, coarse marine algæ of an olive-brown color and leathery substance. In most of these, the thallus shows a distinct differentiation into stipe and lamina, and is attached to the substratum by branched, root-like processes, or rhizoids. This family contains forms which are the largest of Thallophytes, and indeed among the largest of plants.

Not to mention several contributions by earlier writers on the Algæ, three papers have recently been published which deal with the anatomy of Laminariaceæ, all by German writers and including observations on four genera. Reinke has described the anatomy of *Laminaria saccharina*, *L. digitata*, and *Alaria esculenta;* † Will, that of *Macrocystis luxurians ;* ‡ and Grabendörfer, that of *Lessonia ovata.*§

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* The studies whose results are here recorded were carried on in the Cryptogamic Laboratory of Harvard University, under the direction of Dr. W. G. Farlow.

The following are the titles of the preceding papers of this series: —

1. Notes on some Species in the Third and Eleventh Centuries of Ellis's North American Fungi. Proc. Am. Acad, XVIII. 65–85.

2. The Cryptogamic Flora of the White Mountains. Appalachia, III. 232-251.

3. Notes on some Species of Gymnosporangium and Chrysomyxa of the United States. Proc. Am. Acad., XX. 311–323.

4. Notes on Arctic Algæ; based principally on Collections made at Ungava Bay by Mr. L. M. Turner. Proc. Am. Acad., XXI. 469–477.

† Beiträge zur Kentniss der Tange, — Pringsheim's Jahrbücher für wiss. Botanik, Bd. X. p. 371, 1876.

‡ Zur Anatomie von Macrocystis luxurians, — Botanische Zeitung, 42 Jahrgang, p. 801, 1884.

§ Beiträge zur Kentniss der Tange, — Bot. Zeit., 43 Jahrgang, p. 641, 1885.

These papers show that there is a great uniformity in the character and arrangement of the tissues composing the thallus in the different genera.

The adult stipe of the Laminariaceæ may be said, in general, to be composed of four forms of tissue. At the centre is a bundle of twisted and intertangled hypha-like filaments, among which are scattered long trumpet-shaped or funnel-formed cells, placed by twos with their large ends together. Will describes in detail a zone of tissue, composed of sieve-tubes with very abundant sieve-plates, which surrounds this core in the adult stipe of Macrocystis. Outside of the central filamentous tissue, or *medulla*, is a layer of elongated cylindrical cells, whose side walls are very much thickened and abundantly marked with pits, their intercellular spaces being filled by a slimy substance. In Macrocystis and Lessonia, this layer, which may be termed the *inner cortex*, is sharply defined against the medulla; but in Laminaria the medullary filaments penetrate its inner cell-rows, and cause a blending of the two tissues, so that no clear line of demarcation can be fixed between them. Next without the inner cortex are rows of thin-walled cells, more angular than the last in form. These compose a tissue called the *outer cortex.* They become smaller toward the surface of the stipe, and pass gradually into the single superficial layer of cells which forms the *epidermis.* This latter layer consists of small cells whose exposed outer walls are rounded and much thickened, and which contain the olive-brown pigment characteristic of this group of Algæ. The cells of both outer cortex and epidermis are traversed by very delicate partitions, both parallel and oblique to their walls. In Alaria, the cortical tissue seems not to be distinguishable into outer and inner layers.

The lamina is, anatomically as well as morphologically, a terminal expansion of the stipe, and shows the same tissues as the latter, but in quite different proportions. The medulla resembles that of the stipe, but the trumpet-shaped cells seen in the latter are not present. The cell-walls of the inner cortex are thinner than in the stipe, and contain no pits. The outer cortex forms but a small part of the thickness of the blade, and consists of small, thin-walled cells passing into an epidermal layer, as in the stipe. In Alaria, the stipe is prolonged into the lamina as a midrib, whose anatomy is that of the stipe itself.

The rhizoids consist of a mass of cells somewhat thick-walled at the centre, and decreasing in size and thickness of wall toward the surface, the outer layer forming an epidermis. No trace of medulla is

present. At their points of contact with the substratum, the exposed cell-walls become very much, and often irregularly, thickened.

Each of the papers above quoted contributes something in regard to the growth or development of the species treated, and reference may be had to them for full details. It seems well established that the seat of growth in length is the point of union of stipe and lamina, where growth takes place either periodically or constantly. How the stipe grows in thickness seems as yet hardly clear. Reinke describes a "meristem cylinder" composed of the inner cells of the outer cortex in Laminaria, to which he ascribes a function similar to that of the cambium of Dicotyledons. And Will describes a peculiar layer immediately outside of the medullary tissue in the young stipe of Macrocystis, which serves as the source of increase of the medulla; this ring disappears in the adult stipe. The cells of the epidermis increase by the formation of walls at right angles to the surface, thus keeping pace with growth in thickness, as well as in length.

The differentiation of the frond into stipe and lamina is evident from a very early stage. When quite young, the stipe consists of a cortex and a few thick-walled axial cells, from which the medullary filaments develop later, as outgrowths. At a corresponding stage the lamina consists of two large-celled layers and epidermis, although an earlier condition is known in which it is but a single cell thick. The lamina is annually renewed from the region of growth, and Reinke considers it probable that not much change occurs in the structure of a given lamina, but that the successive steps in the development of the complex adult frond from the comparatively simple young condition are accomplished by an increase in complexity of structure in successively formed fronds, until the adult type is reached.

Agarum Turneri.

Agarum Turneri, the so-called Sea Colander, is one of the Laminariaceæ, having a cylindrical stipe 10 to 30 cm. in length, which becomes flat toward its upper end and finally expands into a broad lamina of an ovate or oblong shape, with a strongly cordate base and crisped margin. Through the middle of the lamina passes a broad, flat continuation of the stipe, the midrib. The lamina is abundantly perforated by holes of considerable size, which will be treated in detail later. The stipe splits up, at its base, into numerous branching rhizoids, by which the plant is attached to the substratum. The whole

plant often reaches a meter in length. This alga is an inhabitant of the Arctic Ocean, and extends southward on the western shore of the Atlantic to Boston, and in the Pacific to California and Japan; being, like other Laminariaceæ, a lover of cold waters. At Eastport, Maine, it occurs at low-water mark, but south of that point it grows in deep water, from which it is frequently washed ashore.

At the suggestion of Dr. W. G. Farlow, these investigations have been undertaken with the purpose of learning the anatomy of the adult and of the young frond, and the mode of formation of the perforations of the lamina, in *Agarum Turneri*. The material used has been alcoholic, and has consisted of young fronds collected at Eastport in September, 1877, by Dr. Farlow, and of adult plants obtained at Marblehead, Mass., in November, 1885, by myself.

1. *Adult Anatomy.*

The stipe of the adult frond does not differ in its structure from the general type of the Laminariaceæ. The medulla and inner cortex together compose about one third of the whole diameter of the stipe at its base, but gradually enlarge so that, at the upper end, they form one half of its diameter. The component threads of the medulla are rather loosely matted together, and have many rounded cells scattered among them. These threads penetrate the innermost layers of the inner cortex quite freely, causing a gradual passage from one tissue to another, as in Laminaria. The cylindrical cells of the inner cortex average from three to four times as long as broad. Their side walls are much thickened and pitted, while their end walls are thin. The outer layers belonging to this tissue consist of smaller cells than the rest, and are clearly distinguishable from the inner rows of the outer cortex, which lie next to them. These latter form a darker and more refractive layer of cells, since they are very rich in protoplasm. The cells of the outer cortex become smaller and thinner-walled toward the surface, and their superficial layer is modified to form an epidermis. The epidermal cells have their exposed ends rounded and much thickened, and contain the characteristic olive-brown pigment. As in Laminaria, the cells of the whole outer cortex are crossed by very delicate parallel and oblique walls. The epidermis and cells immediately below present all the appearances of growing tissues.

The midrib continues the tissues of the stipe, but the ratios between the different tissues vary markedly from those in the stipe. The outer

cortex, inner cortex, and medulla occupy about one, five, and two eighths, respectively, of the radius of the midrib. That is, the increase in proportion of the inner cortex, observed in passing upward from the base of the stipe, continues rapidly into the midrib, until the outer cortex is reduced to a very thin tissue. The outer cortex consists of a few layers of thin-walled cells arranged in rows perpendicular to the surface, the exposed outer layer being modified to form an epidermis. The inner cortex forms an abrupt change from the last, consisting of rounded cells with walls not so thick as in the corresponding cells of the stipe, and without pits, but much thicker than those of the outer cortex. The outer cells of this tissue are somewhat smaller than the rest. The medulla is identical in character with that of the stipe, and its filaments penetrate the inner cortex.

The passage from midrib to lamina is rather gradual, and the latter becomes thinner towards its edge. In the lamina, the distinction between outer and inner cortex is lost, and the whole cortical tissue consists of thin-walled cells. The inner layers are rather large-celled, but the outer cells are smaller, while those of the epidermal layer are quite small and have their outer walls thickened. (Fig. 7.) At the margin, where the frond is quite thin, the cortex is reduced to a very few cell-layers. The medulla becomes gradually thinner toward the margin of the lamina, where it consists of a few fine filaments fused into an indistinct mass. The rhizoids originate from the end and lower portion of the stipe, branching freely and irregularly, and often are considerably expanded at the ends which come in contact with and attach the plant to the substratum. Their tissue is a direct continuation of the outer cortex of the stipe, and is similar to it except in being rather smaller-celled. The outer cell-layer forms an epidermis, and the layer which comes into immediate contact with the substratum has its exposed walls thickened very strongly, and often irregularly, so that it not infrequently happens that the cavity of a cell is nearly or even wholly obliterated by the thickening of its wall.

2. *Growth.*

The cambium-like layer described by Reinke for Laminaria has also been mentioned as occurring in Agarum, but there appears to be another region of growth in the latter genus, which includes the epidermis and cell-layers immediately underlying it to the number of two or three. I believe that growth in thickness takes place in these two regions, each meristem adding, by its activity, to the tissue lying within it.

As in Laminaria, the epidermis follows growth in thickness by the increase of its cells through the formation of radially perpendicular walls.

The lamina of Agarum has never been observed to present any evidences of periodic renewal so plainly shown among the species of Laminaria, especially the digitate forms, during the spring months, although it has been collected in great quantities at all seasons on the coast of Massachusetts. This fact and the presence at all seasons of abundantly forming perforations, and only these in the basal part of the lamina, make it quite certain that the growth is continuous.

Even in very young fronds, the exact tip is not known. Many fronds, when very young, are markedly attenuated at the upper end, but none has been found with the actual tip still present. In any but a very early stage, the attenuate character of the upper end is lost and the lamina is ragged and water-worn at its apex.

3. *Anatomy of Young Frond.*

The specimens of the young frond of *Agarum Turneri* which have been at my disposal are as small as any known, but none is sufficiently young to show the one-layered condition of the blade known in Laminaria, if it exists in this species. A young frond of 3 cm. long has a stipe 4 or 5 mm. in length and not over .5 mm. in diameter, with several branched rhizoids at its base. The lamina is oblong in shape, about a centimeter broad and of the thickness of very thin writing-paper, with the margin slightly crisped or wholly flat. Through the middle of the lamina the midrib appears as a rather broad flat band, of about twice the thickness of the lamina and of a firmer texture. (Fig. 1.) Such a frond, while much simpler than in the adult condition, includes all the tissues of the latter except the medulla.

The stipe has an outer cortex composed of radial rows of rather thin-walled cells, the whole of which is deeply colored by the olive-brown pigment. This tissue is covered on its surface by a homogeneous cuticula, in consequence of which the exposed ends of the outer cells, while markedly rounded, are not thickened. The rest of the stipe, composing about two thirds of its diameter, consists of elongated cells closely resembling those of the inner cortex of the adult, but with somewhat less thickened walls. (Fig. 2.) No medullary threads are present at this stage, but a little later, when the perforations of the lamina begin to appear, they may be seen arising as outgrowths from the axial cells of the stipe. The struc-

ture of the midrib agrees completely with that of the stipe. A comparison of the two with the adult shows that the midrib undergoes the less change of structure, remaining always more primitive in condition. The lamina of such a frond as we are considering consists of two or three rows of large, squarish, thin-walled cells, covered by epidermal layers of much smaller cubical cells, containing the brown pigment. (Fig. 3.)

About simultaneously with the appearance of medullary threads in the stipe, they are seen between the large-celled layers at the base of the lamina, and gradually push out towards its edge as the plant grows older. The lamina does not further increase in complication of structure for a considerable time.

4. *Perforations of the Lamina.*

The very young lamina of *Agarum Turneri* is quite imperforate for a time, but when a length of 3 to 4 cm. is attained, the perforations, afterward so characteristic, begin to be formed in all parts of the frond. They continue to be developed during the life of the plant, especially at the base of the lamina and near its middle along the midrib, but also wherever growth has produced a considerable unperforated space. Any large frond will furnish an abundance of perforations in all stages of development. (Figs. 4 and 5.) The rarity of the occurrence of such holes among plants and the evident definiteness of their development give to their study much interest and importance.

The first indication of the formation of a hole is the appearance, on either of the faces of the lamina, of a small, conical, papilla-like elevation, with a corresponding depression on the opposite surface. (Fig. 5, *a*.) This papilla continues to push up until a rupture occurs at its tip (Fig. 5, *b*), and a small opening, like a pin-hole, is made through the lamina (Fig. 5, *c*). If a piece of a lamina with papillæ in various stages of development be held to the light, the tips of the papillæ will be seen to be translucent, and those most so which are nearest the rupturing point. This shows that there is a gradual decrease in the thickness of the tissue at the tip of a papilla, from the time of its beginning until the rupture takes place. The hole now increases in size with the growth of the frond (Fig. 5, *d*), and may reach 2 cm. in greatest diameter and become very irregular in shape, though usually of a broadly elliptical form. It retains for a long time the character of a perforation of the lamina with a raised margin

when viewed from one surface, or a depressed one when viewed from the other face of the lamina.

The microscopic study of the details of the development of the perforations has required only sufficiently thin sections of their various stages. Free-hand cutting wholly failed to give these. A piece taken from the base of a lamina, and containing several early stages in the development of perforations, was then imbedded in paraffine and cut into very thin sections by means of the microtome. The sections were then mounted in balsam, according to the usual method for thin sections of animal tissues. Although this seems a hard treatment for an algal tissue, the tough, leathery character of the frond enabled it to withstand well, and very satisfactory results were obtained.

Sections prepared in this way show that a perforation originates as a depression of the epidermis of either of the surfaces of the lamina. (Fig. 6, *a*.) This depression is formed by a rapid multiplication of the cells of the epidermis in that region and the incurving of the layer in consequence of its increased length. (Fig. 7.) After the inward bending has begun, the continued growth of the epidermis causes it to press deeper and deeper into the underlying tissue, which is compressed and pushed aside, until the medulla is reached. (Fig. 6, *b*.) The depression has, at this stage, the form of a basin with sloping sides and a nearly flat bottom, lined by the unbroken epidermal layer. The bottom of this depression lies against the medullary tissue, while its sides are in contact with the compressed cortex and form angles with the medulla.

Further growth appears to take place only in that portion of the epidermis which forms the sides of the depression, and has two effects. Since the bottom of the depression remains passive while the sides are tending to push on into the medulla, the former is stretched with increasing force until a rupture occurs near its middle; this whole bottom layer of epidermis now dies and falls away, leaving the sides unrestrained. Under the influence of their rapid growth, the latter press into the medulla like a circular punch, cutting out that part of the medulla formerly in contact with the bottom of the epidermal depression. (Figs. 6, *c*, and 8.) Meanwhile, that part of the cortex of the other surface of the lamina which corresponds in position to these lost tissues has begun to show a changed appearance. Although not directly affected by the growth of the depression just described, it becomes dark and shrivelled in appearance (Fig. 8), and soon breaks away from the living cortical tissue surrounding it, escaping into the water as a small block of dead and collapsed cells. (Fig. 6, *d*.)

In this way the perforation of the lamina is completed, and we have a small hole, not usually exceeding .5 mm. in diameter, formed partly by the pushing aside, and partly by the death and breaking away of the tissues which formerly occupied its place.

Where the tissues have been pushed aside by the ingrowing epidermis, they remain covered by it, but where a part of the tissue has died and fallen away, as in case of the cortex of one surface, that part which becomes thus exposed is not at first protected. But very soon the exposed living cells of the cortex begin to thicken their outer walls and to acquire the characteristic brown color of epidermal cells. Finally they become completely indistinguishable from and continuous with the cells of the original epidermal layers of the two surfaces of the lamina. (Figs. 6, *e*, and 9.) In this we have distinct evidence that the epidermis consists simply of modified cortical cells, and that any cortical cells may become epidermal in character, if exposed to external influences.

Further changes in the perforations affect their size only, which increases with the growth of the lamina.

The results of the investigations detailed above may be summarized as follows: —

1. The structure of the adult frond of *Agarum Turneri* agrees closely with that of other Laminariaceæ, and especially with that of Laminaria.

2. The frond of Agarum grows in length at the union of stipe and lamina, as in other Laminariaceæ; growth in thickness probably takes place in two regions.

3. The structure of the young frond is much simpler than that of the adult, but its relations to the latter are clearly traceable.

4. The perforations of the lamina are formed by the simultaneous occurrence of an indentation of one surface and the death of the corresponding part of the opposite surface.

These results will serve at least to call up still unanswered questions, many of which require for their satisfactory solution access to abundant growing material in all stages. For a student who can command these desiderata, the work to be done on the Laminariaceæ is as interesting and important as the many unanswered questions connected with the study of other groups of marine algæ.

EXPLANATION OF PLATES.

Fig. 1. Young frond of *Agarum Turneri* about 3 cm. in length. $\times \frac{5}{2}$.

Fig. 2. Transverse section of stipe of a frond like Figure 1. *cut.* Cuticula. *o. c.* Outer cortex. *i. c.* Inner cortex. $\times$ 175.

Fig. 3. Transverse section of lamina of a similar frond. *e.* Epidermis. *c.* Cortex. $\times$ 175.

Fig. 4. Part of adult lamina, showing perforations in various stages. $\times \frac{1}{2}$.

Fig. 5. Section across basal part of lamina, showing various stages in formation of perforations. *a, b, c, d.* Successive stages. *f.* Plane of lamina. $\times$ 2.

Fig. 6. Diagrammatic view of five successive stages, *a, b, c, d, e,* in the development of a perforation. *c′.* Cortex. *m.* Medulla.

Fig. 7. Detailed figure of an early stage (Fig. 6, *a*) of a perforation. *e.* Epidermis. *c.* Cortex. *m.* Medulla. *x.* Apex of depression. $\times$ 175.

Fig. 8. Detailed figure of a later stage (Fig. 6, *c*). *e, c, m,* as in Figure 7. *x.* Medullary tissue cut out by growth of epidermis. *y.* Fragment of cortical tissue cut out. *z.* Cortex of upper surface beginning to shrivel. $\times$ 175.

Fig. 9. Detailed figure of section through a completed perforation (Fig. 6, *e*). *e, c, m,* as in Figure 7, the right-hand portion being represented in outline only. *v.* Remnants of dead cortical cells. $\times$ 175.

PL. I.

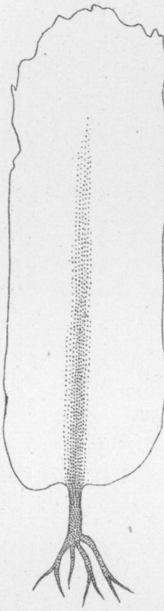
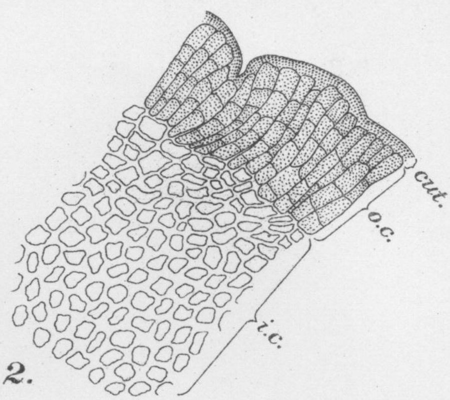


Fig. 1.



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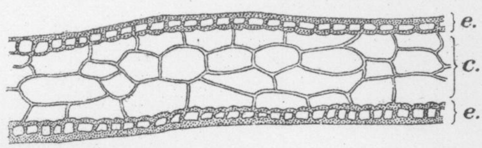

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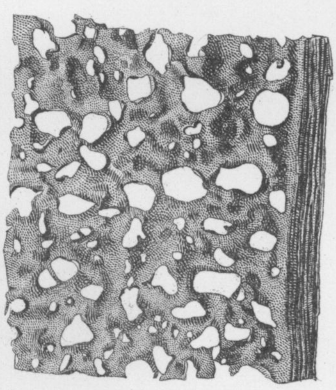

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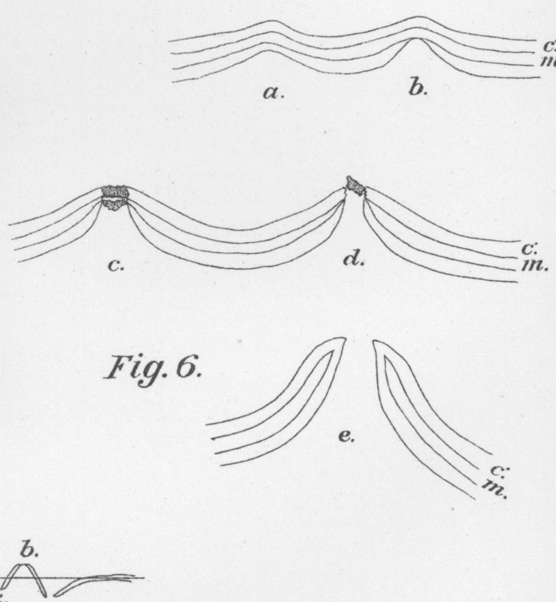

Fig. 6.

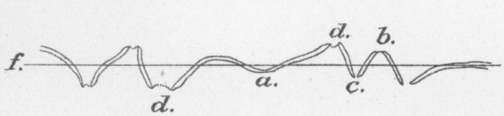

Fig. 5.

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DEVELOPMENT OF AGARUM.

PL.II.

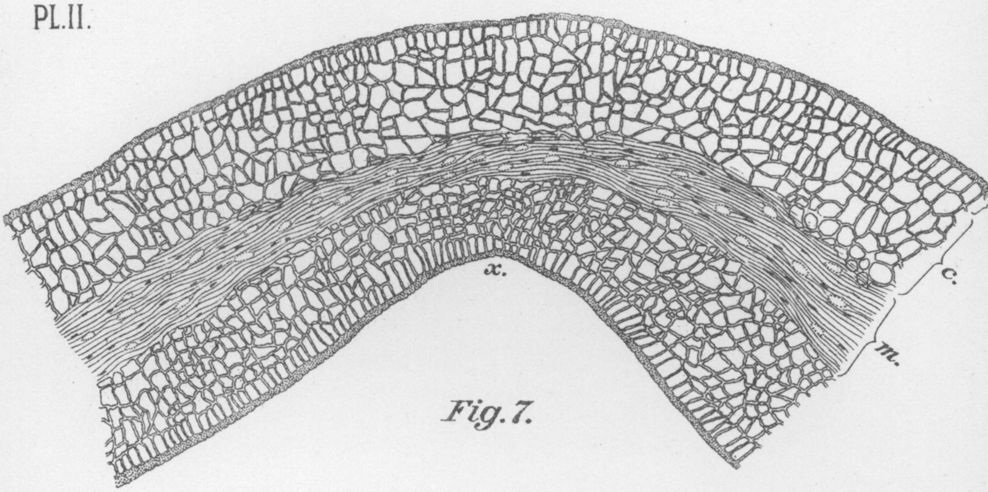


Fig. 7.

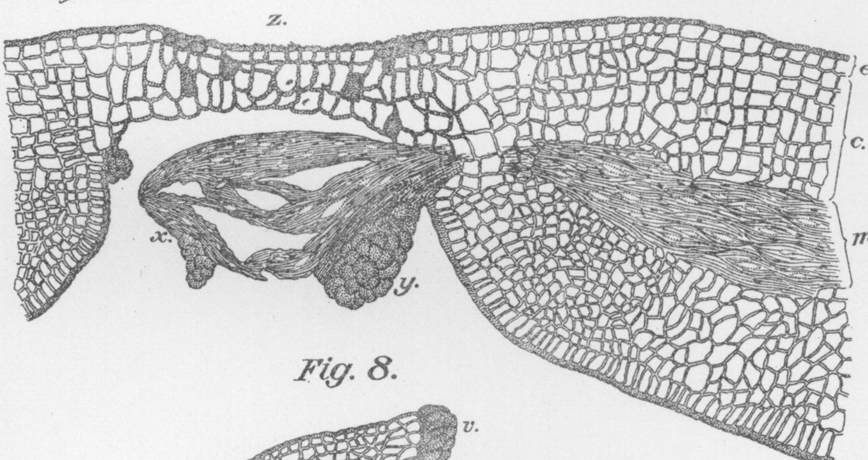
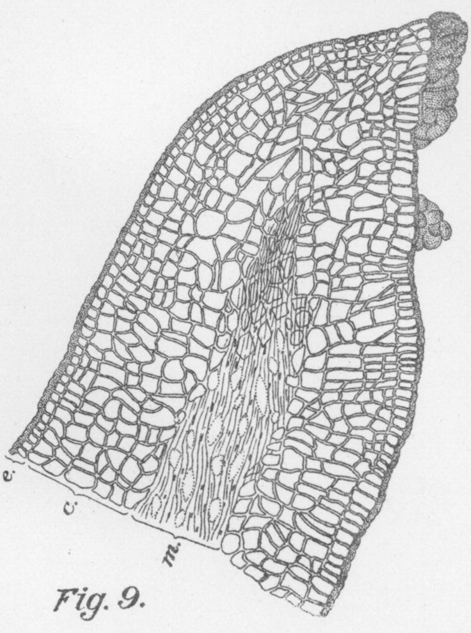


Fig. 8.



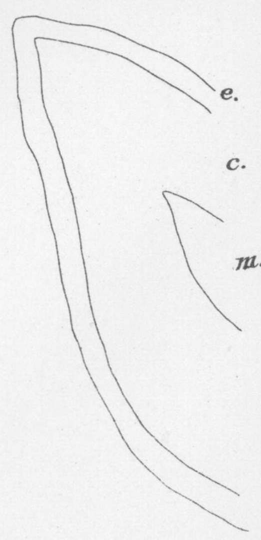


Fig. 9.

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